Review Article

Effects of the Dysregulation in Hedgehog–Gli Singnaling Pathway On the Growth and Metastatic Potential of Human Hepatocellular Carcinoma

Xiuyan Huang,MD, Zili Huang,MS,Hui Wu,MD,Qi Zheng,MD,Xinyu Huang1*, MS

from Department of General Surgery, the 6th People's Hospital of Shanghai, School of Medicine, Shanghai Jiaotong University, Shanghai 200233, PR China(Dr HXyan,Wu,Zheng and HXyu),Department of Radiology, the Central Hospital of Shanghai Xuhui District, Shanghai 200031, PR China(Dr HZL)

ABSTRACT Hedgehog (Hh)–Gli singnaling pathway not only controls the embryonic development, but also plays an important role in tumorigenesis. Gli, a zinc finger transcription factor in the vertebral Hh signaling pathway, combines to the special sequences of distal Hh targeted genes and directly controls the transcription of targeted genes, which played a key role in Hh signaling pathway. Recently, a series of studies have shown that aberrant activation of Hh–Gli singnaling pathway involved in the formation and maintenance of hepatocellular carcinoma (HCC). The blockade of Hh signaling pathway by cyclopamine/KAAD–cyclopamine and/or Gli–siRNA induced reduction of DNA synthesis leading to marked cell growth inhibition and caused significant attenuation in invasiveness and motility of HCC cells. Collectively, Hh signaling activation is an important event for development and invasion of human HCC. Blockade of the Hh signaling pathway may be a potential target of new therapeutic strategy for HCC, and may have potential clinical application.

Key Words: hepatocellular carcinoma;hedgehog singnaling pathway;Gli transcriptional factor; molecular targeted therapy

Introduction

Hedgehog (Hh), a highly conserved secreted glycoprotein encoded by Hh gene, was first identified in a large drosophila screen for genes that were required for patterning of the early embryo by Nüsslein-Volhard C in 1980. Hh signaling is essential for cell differentiation and organ formation during embryogenesis, and it is also required not only for cancer initiation but also for tumor growth [1]. Recently, researchers found that aberrant activation of Hh–Gli singnalling pathway involved in the formation and maintenance of hepatocellular carcinoma (HCC) [2 4].

The authors have no commercial,proprietary,or financial interest in the products or companies described in this article.

Corresponding author:Xinyu Huang ,Tel./Fax: +862164701361,E–mail: h20070629@163.com
Note: X–Y. Huang, Z–L. Huang, and H. Wu contributed equally to this work.

ISSN:1538-5124/$-see front matter

The components and regulation of Hedgehog (Hh)–Gli signaling pathway

Hh-Gli signaling pathway includes three types of Hh signal peptides (sonic hedgehog, SHh; indian hedgehog, IHh; desert hedgehog, DHh), membrane proteins (patched homolog 1, Ptch1; patched homolog 2, Ptch2; smoothened, Smo; human hedgehog inhibitory protein, HHIP; growth arrest-specific gene 1, GAS1), nuclear transcription factors (Gli1, Gli2, Gli3) and downstream target genes (Ptch, Gli1, Bcl2, CCND2, Snail, N-myc, Cyclins, etc.) [5,6]. Gli gene, located at chromosome 12 position (q13 to q14.3), was first identified due to its high expression in glioblastoma by Kinzler KW in 1987 [7]. In the next 10 years, four members (Gli1-4) from Gli gene family were positioned through fluorescence in situ hybridization (FISH). Gli4 was excluded from the family for the similarity in structure with zinc finger protein drosophila kruppel family. Thus, the Gli gene family has actually three members, namely Gli1-3. Researchers also found that SHh signal peptide is widely expressed in mammal Hh signal pathway, Ptch1 is the target gene of Gli2 and N-myc is essential in cell proliferation mediated by SHh[6].

Hh signaling is initiated by the binding of Hh protein (SHh,

IHh, DHh) to its receptor Patched (Ptc), which in the absence of Hh protein represses signal transduction by inhibiting Smo. Binding of Hh protein to the Ptc receptor abolishes the inhibitory effect of Ptc on Smo, and allowing Smo to transduce the signal towards the nucleus via activating the Gli family of zinc finger transcription factors, which start the expression of target genes (Ptch, Gli1, Cyclins, etc.). Evidence suggests that vertebrates have at least three distinct Gli genes, Gli1, Gli2 and Gli3. Gli1 and Gli2 represent the main activators of Hh-target genes, while Gli3 acts mainly as repressor, although some Gli3 activator function is also involved in induction of target gene transcription [8]. It is demonstrated that Gli1 is a stronger activator than Gli2, and Gli2 enhances the transcriptional activity of Gli1 [5,9]. Some results showed that Gli2 plays a predominant role in the proliferation of HCC cells[10]. What merits our particular mention is that Gli1 promotes the transition from epithelial to mesenchymal character (EMT) by up-regulating the expression of Snail, which involved in the increased potential of invasion and metastasis in HCC. Supressor of fused (Sufu) protein down-regulates the transcriptional activity of Gli by which the Hh signaling is inhibited [2,4]. In vertebrates, the binding of HHIP and GAS1 to Hh protein inhibites the binding of Ptch to Hh protein, repressing the Hh signaling pathway endogenously. The formation and maintenance of HCC is related not only to aberrant activation of Hh singnalling pathway but also to the important cross talks between Hh and other intracellular signaling pathways (Ras/MAPK, phosphatidylinositol-3, Wnt, etc.) [11].

Aberrant activation of Hedgehog (Hh)−Gli singnalling pathway in HCC

It is known that Hh-Gli signaling pathway controls formation and differentiation in normal cells and regulates proliferation and metastasis in tumor cells. The malregulation of Hh-Gli pathway helps growth and mantainence of cancers, such as gastric, prostate, and small-cell lung carcinoma [6]. Hh signaling plays a critical role in construction of liver cells during development, while healthy adult livers exhibit little Hh activity. It is demonstrated that Hh is activated in HCC, including SHh, Ptch1, and Gli1; SHh up-regulates the expression of N-myc, Sfrp1, Ptch2, and CyclinD, enhancing the mitosis and proliferation of tumor cells [2,4]. Cheng WT et al. studied by tissue chip and found that the expression of Shh, Ptch1 and Gli2 in HCC tissues were 63.6%, 45.5% and 68.2% respectively, which were higher than those in the adjacent-tumor tissues ($P<0.05$). They also found that overactivation of Gli2 may result in cell malproliferation by up-regulating the expression of Cyclin D1, involving in the formation and development of HCC[12]. Wang XG studied 14 HCC tissue slices and immunochemistry showed that the positive ratio of the components of the Hh signaling pathway Gli, Ptch, Ihh and Smo is 42.9%, 71.4%, 71.4%, and 85.7%, respectively [3]. Guo LM reported that the expression of Shh mRNA was 57.1% (12/21) in tumor tissues, whereas it was 4.8% (1/21) in nontumor tissues[2]. Foreign researches[4,13,14] also showed that the expression rate of Ptch1, Gli1, and SHh is over 50%, 50%, and 60% in HCC respectively, which were higher than those in adjacent-tumor tissues and had no obvious differences in tumor size, gender, differentiation, and liver cirrhosis. Hh pathway expresses and functions in hepatocellular carcinoma (HCC) cell lines, such as Hep3B, Huh7, and PLC/PRF/5, down-regulating the expression of Hh-targer genes to induce apopdosis and to repress cell growth. HCC cell lines, including HCC36 and HepG2, exhibite little expression of Hh-targer gene, by which cell proliferation cannot be inhibited through repressing Hh pathway, which showed that activation of Hh signaling pathway in liver was crucial in human HCC progressing. Further studies are needed to tell whether ectopic expression of Hh and malregulation of Hh pathway in liver could induce HCC.

Recent researches[15,16] show that Gli as the zinc finger transcription effector plays an important role in embryonic differatiation and development. Overexpression of Gli1 induces low metastatic potential cell lines converting to high potential lines, which attracts great attention of researcher in tumorigenesis. Aberrant activation of Hh-Gli singnalling pathway improves proliferation, invasiveness, metastasis potential of HCC cells. Transcription of Gli1 represents the activity of Hh pathway, being critical in many tumor stem cells. Hh-Gli signaling pathway improves tumorigenesis through regulating Wnt, and Notch pathways in tumor stem cell [17]. Surprisingly, a recent study proposes that Hh-Gli signaling acts only on the tumor stroma, therefore, more studies are needed to demonstrate the effect of Hh-Gli signaling on tumor mesenchyma and tumor stem cells [18].

Recent study about the new member of Hedgehog (Hh)−Gli signaling pathway

Metastasis suppressor 1 (MTSS1), the gene encoding MIM putative splice variants, is located in chromosome 8q24, which was first discovered by Kenneth J, Pienta in 2002 through improved mRNA differential display method. It was thought to be an inhibitor in tumor metastasis, while more and more researches showed that the protein “missing in metastasis”, which was known as MIM and coded by MTSS1, has been characterized as an actin-binding scaffold protein that may be involved in cancer metastasis[8,19]. MIM potentiates Gli-dependent transcription, gaining an increasing concern in different aspect of cancer

researches[8]. The mechanism by which MIM enhances Gli-mediated transcription appears to be direct, binding to Gli1 or Gli2 and also to the Gli-complex-associated protein Sufu. It has been proposed that MIM, Sufu and Gli form a ternary complex, which enhances transcription of Shh-responsive genes. A study found that MIM was able to potentiate transcription in a variety of cell types and systems. Thus, MIM is likely a general SHh target, which may participate in tumor development and progression via the SHh pathway. Confirmation of this hypothesis waits further testing in other tissue types and systems[5,8,9]. MIM proteins are important regulators in controlling cell growth and development. The accumulating evidence suggested a role of MIM-B, human MTSS1 gene coded protein, in carcinogenesis, yet its role in the development of hepatocellular carcinoma has not been examined thus far. So it is worthy of effort to explore the regulation mechanism of MIM-B in HCC for this potential theraputic target[20]. A recent domestic study shows that the splicing-specific novel proteins encoded by the 2.2 kb singly (termed as TPss)and doubly(termed as TPds) spliced variants of HBV improve Huh7 cells highly express MTSS1, which disturb cell function including the reorganization of the cystoskeleton and metabolism,and attribute to the abnormal cell proliferation,mobility and aberrant cellular metabolism[21]. It has not been reported that whether MIM activates Hh to improve HCC progressing. We found up-regulated expression of MIM-B and overactivation of Hh-Gli signaling pathway in HCC tissues in our previous work. However, we are still working on the relationship between them.

Prevention and treatment via Hedgehog (Hh)–Gli signaling pathway

Many experimental interventions have been performed on different signaling pathways today [22 24]. However, we still cannot find the specific medications for Hh signaling pathway. Cyclopamine, a teratogen isolated from the corn lily, is a naturally-occurring chemical that belongs to the group of steroidal jerveratrum alkaloids. Studies suggest that cyclopamine acts as a primary inhibitor of the Hh singaling pathway by influencing the balance between the active and inactive forms of the Smo in cells, which provides a potential treatment for certain cancers in which Hh is overexpressed[25]. But inhibitors such as cyclopamine only work when the overexpression of Hh is caused by the change of upstream gene in Hh signaling pathway. Recent study shows that cyclopamine may not affect tumor cells but adjecent mesenchyma cells to suppress Hh, which supports the hypothesis of crosstalk between tumor cells and mesenchyma cells [18].

As final regulators of the pathway, it has been reported that each Gli (Gli1, Gli2, or Gli3) displays unique and sometimes overlapping roles in modulating the SHh pathway in a manner that is cell type or developmental stage dependent. Theoretically speaking, treatment focusing on Gli is of great value because it may suppress HCC growth by cutting off the Hh signaling pathway thoroughly, which means Gli-siRNA has a bright future in finding the cure for HCC[15]. RNAi technology has achieved great results in the experimental studies. Studies in vivo and in vitro show that down-regulation of Gli1, and Gli2 by siRNA resulted in the inhibition of cell proliferation in HCC[10]. Gli1-siRNA completely blocked Gli1 expressions in Huh7 cells. It decreased cell viability to 88.3% after 24 hours and increased 2-fold caspase3 activity after 48 hours in Huh7 cells, which indicates that Gli-1 siRNA reduced Huh7 cell viability through apoptosis induction. Gi1-siRNA also enhanced 5-Fu-induced apoptosis in Huh7 cells[9,15]. In the absence of Hh signaling, Gli1 is transcriptionally silent but Gli2 and Gli3 can be expressed[26], because Gli1 and Gli2 represent the main activators of Hh-target downstream genes, while Gli3 acts mainly as repressor. When applying the upstream inhibitors such as KAAD-cyclopamine to Hh signaling pathway, we should combine the use of downstream inhibitors such as interference of Gli1 and Gli2. Given the high degree of homology between Gli1 and Gli2, we reasoned that a potential Gli1 inhibitor would also target Gli2. GANT61 was able to efficiently block Gli1 as well as Gli2-induced transcription. But inhibition of Smo activity by cyclopamine treatment in low-Gli1 HCC cell line HepG2 had only marginal effects on cell proliferation (0–12% inhibition)[25]. Gli target gene treatment is now in the experimental stage. An announcement has just been made recently that a hedgehog pathway inhibitor from Genentech had shown activity in a phase I cancer clinical trial[27], which indicates a potential wide span application of these inhibitors. Another study found that angiogenic factors, such as VEGF and angiopoietins, were upregulated by SHh, thereby promoting angiogenesis and tumor metastasis. Altogether, joint use of Hh pathway inhibitors and chemotherapy or biotherapy (such as interferon) will be a promising future for the treatment of HCC[15,28].

The fact that Hh signalling can be blocked at different levels is encouraging and might result in improved treatment options for tumors which depend on sustained Hh activity. Further clinical studies are needed to establish more efficient inhibitors which are suitable for therapeutic use[14]. Smo specific inhibitors (cyclopamine/KAAD-cyclopamine) and interference from lentivirus-induced siRNA indicated that acitivity level of Hh was involved with severity and malignant behavior of cancers. Hh can be monitored as a biomarker for tumor progression and a response for drug treatment. However, many aspects of Hh signaling

remain incompletely understood. Further research is needed in multiple areas, including the study of Hh target gene responses, which is required to understand in detail how the graded Hh signals are converted to discrete cell-fate decisions, and to decipher the molecular mechanisms that underlie the exquisite specificity of cellular responses to Hh. In addition, the therapeutic potential of Hh pathway agonists and antagonists in human degenerative diseases and cancer should be further investigated [6].

Conclusion

The fact that the Hh pathway is active in human HCC suggests a significance of novel targeted strategy for HCC therapy. Hh pathway activation strongly reflects the biological aggressiveness of HCC and plays a vital role in its proliferation and invasiveness. Blocking the Hh signaling pathway remarkably decreased HCC cell growth and motility, which indicates it could provide a new therapeutic option for HCC to improve prognosis. However, there are still a lot of unknown aspects in the research of Hh-Gli signaling pathway, such as functions and structures of other components (such as MIM) in the Hh pathway; identification of variant cells after the enhancement of the signal; toxic, side-effect and tolerance of cyclopamine/KAAD-cyclopamine in human body; safety problems of combination intervention of Gli1, Gli2 etc. With improvement of some molecular biotechnologies and innovation of new techniques (cDNA microarray, RNAi, etc.), the Hh-Gli signaling pathway and its cross talks with other pathways will be revealed, which will help us to understand the pathogenesis of HCC and provide us new strategy for HCC therapy [11, 16, 29, 30].

References

1.Pasca di Magliano M, Hebrok M. Hedgehog signalling in cancer formation and maintenance. Nat Rev Cancer, 2003; 3: 903–911.

2.Guo LM, Tang R, Zhong CH, Zhang Y, Lin M, Yu HK. Expression of hedgehog pathway in hepatocellular carcinoma cell lines and in liver cancerous tissues. J Clin Hepatol, 2009; 12: 5–7, 13.

3.Wang XG, Wang WM, Wu WH, Yang YM, Liu YC, Wan YL. Role of hedgehog signaling pathway activation in hepatocellular carcinoma formation. Chin J Clinicians (Electronic Version), 2008; 2: 132–141.

4.Omenetti A, Diehl AM. The adventures of sonic hedgehog in development and repair. II. sonic hedgehog and liver development, inflammation, and cancer. Am J Physiol Gastrointest Liver Physiol, 2008; 294: G595–598.

5.Kasper M, Regl G, Frischauf AM, Aberger F. GLI transcription factors: mediators of oncogenic hedgehog signaling. Eur J Cancer, 2006; 42: 437–445.

6.Varjosalo M, Taipale J. Hedgehog: functions and mechanisms. Genes Dev, 2008; 15: 2454–2472.

7.Kinzler KW, Bigner SH, Bigner DD, Trent JM, Law ML, O'Brien SJ, et al. Identification of an amplified, highly expressed gene in a human glioma. Science, 1987; 236: 70–73.

8.Callahan CA, Ofstad T, Horng L, Wang JK, Zhen HH, Coulombe PA, et al. MIM/BEG4, a sonic hedgehog-responsive gene that potentiates gli-dependent transcription. Genes Dev, 2004; 18: 2724–2729.

9.Chen XL, Cao LQ, She MR, Wang Q, Huang XH, Fu XH. Gli–1 siRNA induced apoptosis in Huh7 cells. World J Gastroenterol, 2008; 14: 582–589.

10 .Kim Y, Yoon JW, Xiao X, Dean NM, Monia BP, Marcusson EG. Selective down-regulation of glioma-associated oncogene 2 inhibits the proliferation of hepatocellular carcinoma cells. Cancer Res, 2007; 67: 3583–3593.

11. Mishra L, Banker T, Murray J, Byers S, Thenappan A, He AR, et al. Liver stem cells and hepatocellular carcinoma. Hepatology, 2009; 49: 318–329.

12.Cheng WT, Tian DY, Xu D. Determination of expression of Hedgehog signaling in hepatocellular carcinoma by tissue chip. Chin J Int Trad Western Med Liver Dis, 2008; 18: 153–155.

13.Sicklick JK, Li YX, Jayaraman A, Kannangai R, Qi Y, Vivekanandan P, et al. Dysregulation of the hedgehog pathway in human hepatocarcinogenesis. Carcinogenesis, 2006; 27: 748–757.

14.Robbins DJ, Hebrok M. Hedgehogs: la dolce vita workshop on hedgehog-gli signaling in cancer and stem cells. EMBO Rep, 2007; 8: 451–455.

15.Wang Q, Huang S, Yang L, Zhao L, Yin Y, Liu Z, et al. Down-regulation of sonic hedgehog signaling pathway activity is involved in 5-fluorouracil-induced apoptosis and motility inhibition in Hep3B cells. Acta Biochim Biophys Sin (Shanghai), 2008; 40: 819–829.

16.Cheng WT, Xu K, Tian DY, Zhang ZG, Liu LJ, Chen Y. Role of Hedgehog signaling pathway in proliferation and invasiveness of hepatocellular carcinoma cells. Int J Oncol, 2009; 34: 829–836.

17.Ruiz i Altaba A. Therapeutic inhibition of hedgehog-gli signaling in cancer: epithelial, stromal, or stem cell targets? Cancer Cell, 2008; 14: 281–283.

18.Yauch RL, Gould SE, Scales SJ, Tang T, Tian H, Ahn CP, et al. A paracrine requirement for hedgehog signalling in cancer. Nature, 2008; 455: 406–410.

19.Machesky LM, Johnston SA. MIM: a multifunctional scaffold protein. J Mol Med, 2007; 85: 569–576.

20.Ma S, Guan XY, Lee TK, Chan KW. Clinicopathological significance of missing in metastasis B expression in hepatocellular carcinoma. Hum Pathol, 2007;38: 1201–1206.

21.Chen WN, Guo DH, Chen JY, Lin WS, Lin JY, Lin X. Effects of the splicing-specific novel proteins encoded by the 2.2 kb spliced variants of hepatitis B virus genome on gene expression profile in Huh7 hepatocytes. Chin J Path Bio, 2008; 3: 321–5, 329.

22.Li Z, Liu CP, Zhang N, Tian Y, Huang T. Dynamics of Wnt signaling pathway in the process of rats carcinogenesis of breast cancer. Chin J Cancer Prev Treat, 2008, 15: 401–403.

23.Yin SC, Zhou XH, Peng T, Li J, Jin J. Expression of insulin-like growth factor-1 receptor, activation of PI3K /AKT and ERK /MAPK signal transduction pathway in nasopharyngeal cancer cells. Chin J Cancer Prev Treat, 2009; 16: 44–47.

24.Li BH, Yang XZ, Li PD, Yuan Q, Liu XH, Gerhard GS, et al. Gene expression regulated by IL-4/stat6 signaling pathway in breast cancer cells with different stat6 activation phenotypes. Chin J Cancer Prev Treat, 2009; 16: 161–163.

25.Lauth M, Bergström A, Shimokawa T, Toftgård R. Inhibition of Gli-mediated transcription and tumor cell growth by small-molecule antagonists. Proc Natl Acad Sci USA, 2007; 104: 8455–8460.

26.Ruiz i Altaba A, Mas C, Stecca B. The Gli code: an information nexus regulating cell fate, stemness and cancer. Trends Cell Biol, 2007; 17: 438–447.

27.Garber, K. Hedgehog drugs begin to show results. J Natl Cancer Inst, 2008; 100: 692–697.

28.Nagase T, Nagase M, Machida M, Fujita T. Hedgehog signalling in vascular development. Angiogenesis, 2008; 11: 71–77.

29.Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology, 2008; 48: 1312–1327.

30.Huang XY, Huang ZL, Tang ZY, Zheng Q, Ye SL. Research on the effect of missing in metastasis in cancer progression. Tumor, 2010; 30: 170–172.